parasites; environmental samples which may be man-made or natural and may contain mixtures of prokaryotic and eukaryotic organisms; or even spliced genomes from a plurality of organisms. Heterologous DNA can further include libraries of DNA, including genomic libraries.

[0020] The vector also includes left and right *Agrobacterium* T-DNA border sequences flanking the unique restriction endonuclease cleavage site (Peralta and Ream 1985). These border sequences allow the introduction of heterologous DNA located between the left and right T-DNA border sequences into a host cell when using *Agrobacterium*-mediated DNA transfer.

[0021] Stable maintenance of high molecular weight DNA in *Escherichia coli* and *Agrobacterium tumefaciens* is made possible because these high molecular weight DNA sequences are carried on a single copy plasmid. With multiple copies of such large DNA inserts, the plasmid might be unstable and if so, the host cells would not be useful for non-plant host cell transformation. This is especially true in cases where the heterologous DNA may encode proteins that are themselves somewhat toxic to the host cell, or in which the heterologous genes' products are toxic, causing selection pressure for loss of the plasmid. Keasling (1999) points out that single-copy plasmids can have the advantage of controlled gene expression and low metabolic burden on the host, but that few such vectors are currently available.

[0022] In one embodiment of the present invention, in a vector, designated the BIBAC vector, the unique restriction endonuclease cleavage site is a BamHI cleavage site. This cleavage site is located between the *Agrobacterium tumefaciens* T-DNA border sequences, as is a selection marker for incorporation of heterologous DNA into the vector (the sacB gene). The BamHI cleavage site and the sacB gene are located such that when heterologous DNA is inserted into the BamHI site the sacB gene is inactivated. The BIBAC vector includes the F origin of

replication from *Escherichia coli* for maintaining the heterologous DNA as a single copy in *Escherichia coli* (Low 1972), and the Ri origin of replication from *Agrobacterium rhizogenes* for maintaining the heterologous DNA as a single copy in *Agrobacterium tumefaciens* and related species. The left and right T-DNA border sequences in the BIBAC vector are derived from the TL-DNA of the octopine plasmid pTiA6.

[0023] The BIBAC vector also includes a selection marker for introduction of the heterologous DNA into bacterial cells such as *Escherichia coli* and *Agrobacterium tumefaciens*. The bacterial selection marker comprises the kanamycin resistance gene. The original BIBAC vector included a selection marker for introduction of the heterologous DNA into a plant cell. In some non-plant host species, appropriate virulence genes may result in transfer of the DNA between the T-DNA border sequences in host chromosome or extrachromosal elements. In cases where T-DNA transfer is desired, the selection marker must be located between the left and right T-DNA border sequences. The selectable marker must be chosen based on the particular host cell. Markers resulting in complementation of auxotrophic mutations are commonly used in yeast; a kanamycin resistance gene conferring resistance to G418 is commonly used in other eukaryotes (see further below).

[0024] The BIBAC vector also includes an origin of conjugal transfer (the oriT origin from plasmid RK2). This origin allows the transfer of heterologous DNA from an *Escherichia coli* host cell directly to an *Agrobacterium tumefaciens* host cell and cells of related species by bacterial conjugation.

[0025] The vector designated BIBAC has been deposited as the plasmid designated pCH23 in the *Escherichia coli* strain designated DH10B (pCH23) pursuant to, and in satisfaction of, the

requirements of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure, with the American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, Maryland 20852 under ATCC Accession No. 69743.

[0026] The vector is used in the method of the present invention to introduce heterologous DNA into a host cell. Accordingly, the vector preferably includes heterologous DNA inserted into the unique restriction endonuclease cleavage site. DNA is inserted into the vector using standard cloning procedures readily known in the art. This generally involves the use of restriction enzymes (in the case of BIBAC, BamHI) and DNA ligases, as described by Maniatis et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York (1982). The vector can then be used to transform a non-plant host cell, such as prokaryotic host cells, e.g. *Escherichia coli*, *Agrobacterium species, Rhizobium species*, other related bacterial species, yeast, filamentous fungi, insects and/or a mammalian host cell.

[0027] An alternative to the standard method of isolating genomic DNA from cultured organisms is to use amplification methods on DNA extracted from nature. Methods have been established to perform polymerase chain reaction (PCR) and obtain large segments of amplified DNA. Commercial kits are available that can result in amplification of DNAs of length up to 35 kb (see www.stratagene.com). Such DNAs can be incorporated into the BIBAC for heterologous expression.

[0028] Heterologous expression of DNA is often most efficient when promoter sequences from the host organism are used to control expression. Such sequences could be cloned into the